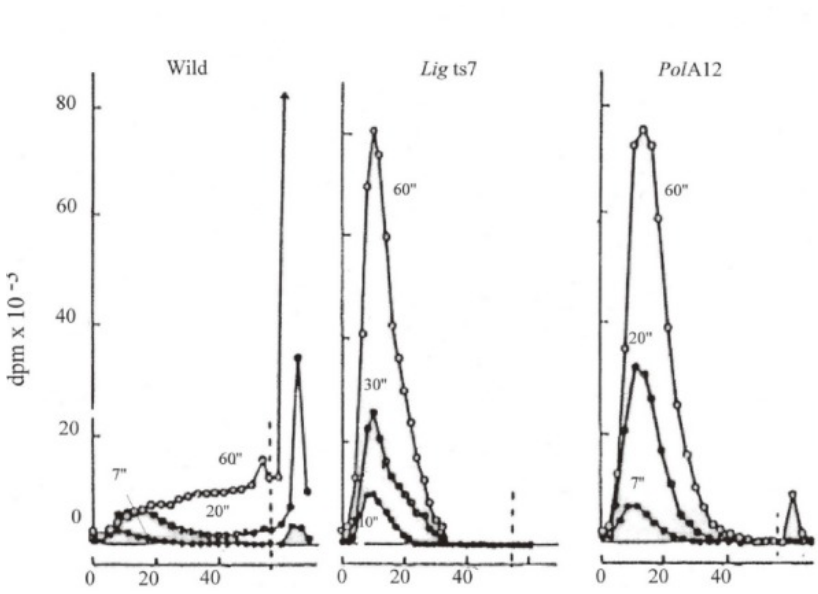
Discussion Worksheet – Fall 2025 - Week 5 (DNA Replication)
Group1

In this experiment a scientist incubated bacteria with radiolabeled dTTP and after specific times of radiolabeling (indicated by 7", 20" etc..= 7 seconds, 20 seconds), he extracted DNA and measured the radioactivity incorporated in DNA (dpm, y-axis) as a function of the size of the DNA molecules (x-axis, arbitrary units).

In the first experiment (left figure) he used a normal bacterial strain (=Wild).



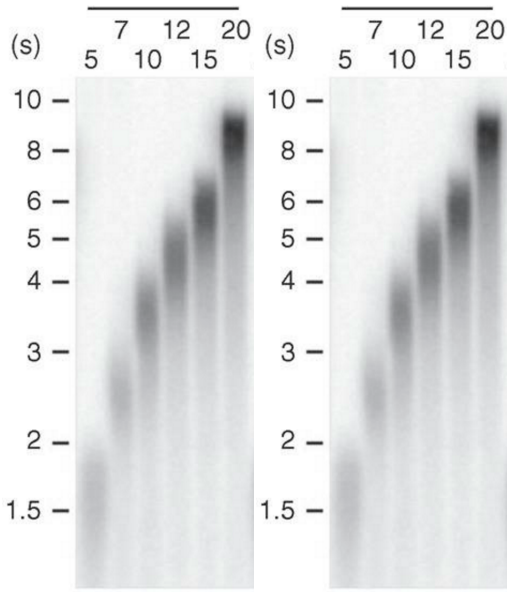
A - Describe the change in size in DNA fragments as a function of time in this strain and explain what the changes in size correspond to.
At 7 seconds, two peaks are detected, one of small size (10 units), one of larger size. At 20 minutes, both of these peaks increase in abundance especially the larger one. At 60 minutes the smaller peak is no longer detected, instead there is mainly a peak of large size very abundant. Based on this experiment, it seems that the small fragments produced early on are converted into longer ones. The smaller fragments likely correspond to the Okazaki fragments which are then processed into longer DNA fragments.

B - In the second and third experiment he used bacterial strains which contain mutations in gene encoding DNA ligase (Lig ts7) or DNA Polymerase I (PolA12).

Describe the differences in DNA synthesis in these strains compared to the wild-type and what this experiment tells us about the roles of DNA ligase and DNA polymerase I in replication.
In both of these mutants, the peak of small size (10 units) accumulate in abundance over time and they are not converted into longer fragments, as opposed to what is seen in the WT (although there is a small amount of longer DNA produced in the PolA12 mutant at longer time, but very little compared to WT). These experiments provide us with the experimental evidence that the activities of DNA ligase and DNA polymerase I are required to process the Okazaki fragments into longer DNA strands (the experiment do not tell us what biochemical activities are involved for each of these enzymes.

Group2

In this experiment, scientists study the properties of bacterial replisomes containing 2 (DiPol.III) or 3 (TriPol.III) DNA polymerases. In a first experiment they incubate each replisome with a primer and a single stranded DNA template in vitro and study DNA polymerization as a function of time.
The size of DNA fragments is shown on the y-axis in kilobases, after incubation with the replisomes for 5,7,10,12,15 or 20 seconds (times are shown at the top of each lane).

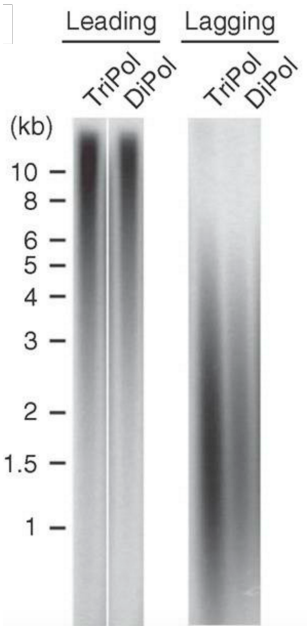


Di-Pol.III **Tri-Pol.III**

- Describe the results observed; what property of Di and Tri-Pol.III replisomes can be concluded from the results of this experiment?

The size and abundance of the DNA products generated during the time course is identical for both replisomes – there is no detectable difference. This means that the activities of the Di/TriPol III replisomes are identical when they polymerize DNA on a simple primer-template substrate in vitro.

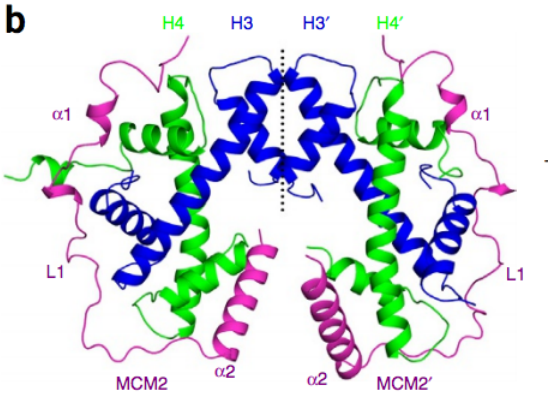
Next, researchers specifically analyze the products generated during replication in vivo on the leading and lagging strands with Di or Tri-Pol.III. The size distribution and abundance of the fragments obtained after incubation of Di or Tri-Pol III with leading or lagging strand templates is shown below.



- Describe the results obtained in this experiment; based on these results and on the results obtained in the first experiment, compare the ability of Di-Pol.III and Tri-Pol.III replisomes to replicate DNA.
The products obtained on the leading strand are identical for the TriPol and DiPol replisomes. However the products obtained for the lagging strands are less abundant for the DiPol than for the triPol (and maybe shorter as well). This means that a Tri-Pol. Replisome is more effective at replicating the lagging strand (but not the leading strand for which both replisomes are as effective).

Group3

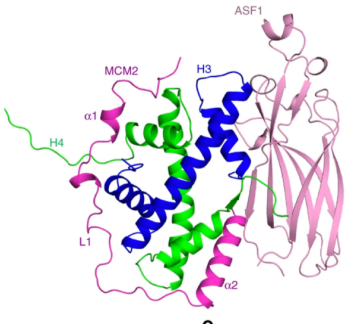
Structural Biologists have studied the structure of the MCM2 protein (part of the MCM protein complex present in the replisome in eukaryotes. They have obtained the structure of MCM2 interacting with the H3 and H4 histones as shown in the structure on the right.



H4' means that a second molecule of histone H4 is present. Same for H3 and MCM2.

The formation of the dimer of this structure is an artefact of structure determination. For the purpose of this problem, only consider that one MCM2 interacts with one H3 and one H4.

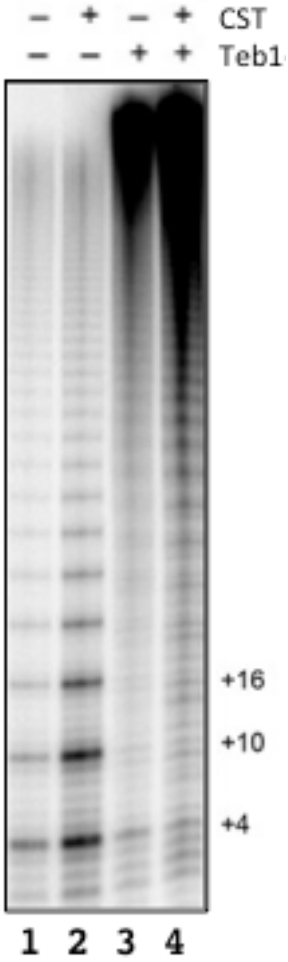
A – What does the dotted line represent?
It's an almost perfect dyad axis (axis of symmetry of 180°). It's not perfect because some parts of the structure don't align when rotated by 180°along that axis.
B- based on what we covered in class, what is the known function of the MCM complex that MCM2 is involved in?
It is the helicase that unwinds the two DNA strands at the eukaryotic replication fork.
C- Based on the structure shown above, what other function does this structure suggest for MCM2 during eukaryotic DNA replication?
MCM2 interacts with histones H3 and H4. Since the MCM helicase encounters nucleosomes and histones when it unwinds the parental DNA during replication, the binding of H3 and H4 by MCM2 at the replication fork might facilitate the recycling of parental histones H3 and H4.



The researchers have now obtained a second of structure of MCM2 bound to histones H3 and H4 in complex with ASF1. The structure is shown on the right.
D- Describe globally the protein-protein interactions that exist in this complex. Does the presence of ASF1 change the structures of H3/H4?
MCM2 interacts with histones H3 and H4 in a manner similar to that seen on the structure without ASF1. ASF1 binds mostly to H3 and to a small region of H4 but on the side of H3 not bound by MCM2.
E- Based on the two structures shown and what you know about the role of the MCM complex during replication, propose a more specific role for MCM2 during eukaryotic replication.
After unwinding the parental DNA strands, MCM2 recovers the parental histones H3 and H4 and 'hands them over' to ASF1, which will then initiate the process of depositing the parental H3 and H4 histones onto the newly replicated strands. So MCM2 serves as a histone chaperone and assists the ASF1 H3/H4 histone chaperone in the mechanism of recycling.

Group4

The purified telomerase enzyme is incubated with a 12nt primer made of telomeric repeats and radio-labeled dNTPs. Two additional proteins Teb1 and/or CST are added(+) or omitted(-) from the reaction accordingly. The results are run on an autoradiography gel. Numbers on the right indicate the numbers of nucleotides added to the unextened primer, which is not shown but would be at the bottom of the gel.



A – In the lower part of the gel for lanes 1 or 2, count the number of faint bands between each more intense band. What molecular events related to telomerase activity do the faint and more intense bands represent?

There 5 faint bands between each more intense band. Each faint band represents the extension of the telomeric DNA by 1 nucleotide. Each intense band represents the extension of the telomeric DNA by one telomeric repeat = 6 nucleotides.

B – What is the effect of the CST protein on telomerase activity especially related to the presence of faint vs. more intense bands? Justify your answer by comparing the patterns observed in lanes 1 and 2.

Addition of CST increases the intensity of the bands but does not change the pattern/relative intensity of faint and intense bands. CST increases the activity of the telomerase enzyme.

C - What is the effect of the Teb1 protein on telomerase activity? Justify your answer by comparing the patterns observed in lanes 1 and 3.

Addition of Teb1 results in a disappearance of the faint/intense bands at the bottom of the gel and production of large extension products migrating at the top of the gel. Teb1 increases processivity of the telomerase enzyme by since the abundant short extension products are no longer detectable.

D - How does adding both Teb1 and CST affect telomerase activity? Justify your answer in one or two sentences.

The activity and processivity are increased by adding both proteins, as the production of longer extension products is enhanced compared to adding either Teb1 or CST alone.